PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 8 August 2003

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
A
19 JUL 2002
RECEIVED BY FAX

The Patent Office

19JUL02 E734745-1 D02806
P01/7700 0.00-0216847.4

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — JED1044

2. Patent (T...) — 0216847.4 — 19 JUL 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — Astron Clinica Limited
The Mount
Toft
Cambridge
CB3 7RL

Patents ADP number *(if you know it)* — 7891427002

If the applicant is a corporate body, give the country/state of its incorporation — England

4. Title of the invention — Method and Apparatus for Investigating Skin Histology

5. Name of your agent *(if you have one)* — Barker Brettell

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — St John's Innovation Centre
Cowley Road
Cambridge
CB4 0WS

Patents ADP number *(if you know it)* — 7442494004

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of Filing *(day/month/year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d)) — YES

Patents Form 1/77

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	5 + 5
Claim(s)	1
Abstract	
Drawing(s)	2 + 2

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature: Barker Brettell — Date: 19 July 2002

12. Name and daytime telephone number of person to contact in the United Kingdom: Barbara Wright — Tel: 0121 456 1364

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 01645 500505
b) Write your answers in capital letters using black ink or you may type them.
c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.
e) Once you have filled in the form you must remember to sign and date it.
f) For details of the fee and ways to pay please contact the Patent Office.

Method and Apparatus for Investigating Skin Histology

Field of the Invention

The present invention relates to a method and apparatus for investigating the histology of skin to provide an analysis of the skin which is independent of the amount of dermal melanin.

Non-melanoma skin cancer accounts for 90% of skin cancers. Within the grouping of non melanoma skin cancer there are two pre-dominant forms Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC) with approximately 75% being BCC's and 20% being SCC's: indeed BCC is not only the most common form of skin cancer it is also the most common form of cancer in humans; it is estimated 1 in 3 Americans will develop a BCC during their life time.

Both forms of cancer are believed to be linked to Ultra Violet exposure causing damage to the DNA of cells existing within the upper layers of the skin. The cancers typically cause local destruction of tissue but although they have the power to metastasise the percentage chance of metastasis is far lower than for melanoma, the more aggressive form of skin cancer.

A large number of different treatment options are now available for non-melanoma skin cancer ranging from surgical excision to light activated drugs that destroy the tumour, to locally applied cryotherapy. The decision on which treatment option is the most suitable depends largely on at which stage the cancer is in its life cycle and the site of the tumour. Both BCC's and SCC's begin life with the tumour cells confined to solely to the epidermis – SCC's are commonly called Actinic Keratosis at this stage – a stage at which they are histologically referred to as "superficial". The cancer can then penetrate and populate the dermis at which point a histologist would refer to them as "infiltrating" or "invasive". Non-surgical treatment has been shown to be effective for treating superficial cancers but is far less effective for infiltrating or invasive cases when surgery is the best option. There are many reasons to prefer a non-surgical intervention namely a better cosmetic result is often achieved and the treatment can be applied at a primary care level – something which is important when the large numbers of these cancers are considered. However, it is also not desirable to treat invasive non-melanoma cancer in such a manner as there is a possibility that not all the cancer will be destroyed therefore requiring surgery at a later stage.

Currently there is no reliable method available to assess whether such a cancer is superficial that can be applied widely enough to reach practising dermatologists and general practice. Confocal microscopy can be used to view the malignant cells and indeed assess whether they are intra-epidermal or not but both the high cost and time required to assess a patient have so far confined its use to research institutions. A useful tool would therefore be one that is both effective in distinguishing superficial from infiltrating and invasive non-melanoma skin

cancer and which is also applicable to a primary care setting.. Skin can be considered to be a layered structure with the epidermis lying over the dermis. The junction between the two layers is called the dermo-epidermal junction and anchored to this layer are cells called melanocytes that produce the pigment melanin. It is these melanocytes which dictate the colour of our skin with black skin having the same number of melanocytes as white skin but the production of melanin being higher. The melanin produced is taken up by keratinocytes in the epidermis which migrate to the surface before flaking and being discarded. The dermis, in contrast, is formed largely from collagen fibres which are tightly bound together and blood vessels.

It has been found that the structure of tissue can be analysed to investigate the presence of chromophores in the tissue by illuminating the tissue with light and then analysing the proportion of light remitted by the tissue. Examples are described in our previously published applications WO98/22023 and WO00/75637. Optically both layers exhibit markedly different properties most notably in the amount to which they scatter light. The epidermis is a low scattering regime in contrast to the dermis where the collagen fibres are on a comparable scale with the wavelengths of visible and near infrared light resulting in a strong interaction and high scattering.

Light striking the outer layer of the skin therefore first has to traverse the epidermis suffering absorption from any pigments, typically melanin, being present. The low scattering nature of the epidermis will ensure that any remaining light enters the dermis with absorption occurring from the collagen fibres and any haemoglobin present. The high scattering nature of the dermis will then return a proportion back into the epidermis which it will travel through again before being remitted from the tissue.

Summary of the Invention

According to the invention there is provided a method for monitoring the presence of chromophores in a sample of skin, the method comprising:
illuminating an area of skin by projecting light of at least two different wavelengths λ_1, λ_2 from a light source, ideally choosing wavelengths where the effects of absorbsion by components other than melanin and collagen, such as haemoglobin, are small compared to the differences produced through variations in collagen, and ideally that the difference between the two wavelengths is maximised,
and receiving light remitted by the illuminated area of skin at a photoreceptor;
analysing the received light to identify and measure the proportion of light of each wavelength remitted from the skin $I_r(\lambda)$;
calculating the ratio of light at each wavelength returned from the skin $R_i(\lambda)$,
calculating the ratio $G(\lambda_1, \lambda_2)$ of the natural logarithms of $R_i(\lambda)$ for each wavelength λ_1, λ_2 and calculating the exponent of $G(\lambda_1, \lambda_2)$ to provide

$$Z = e^{G'} = R_d(c,h,\lambda_1) - R_d(c,h,\lambda_2)$$

which is indicative of the difference between the proportion of light returned from the dermis of each wavelength which is independent of the amount of dermal melanin.

The benefits of this measurement technique are that measurements at just 2 wavelengths are required, the calculation is simple, the method is tolerant of measurement noise and calibration errors, it eliminates the effects of epidermal melanin which is the major absorber in the skin, and it is sensitive to small differences in collagen.

The invention also provides apparatus for analysing skin in accordance with this method.

If the light striking the tissue is described as $I_0(\lambda)$ where λ refers to the wavelength of light, absorption due to melanin as $A(m,\lambda)$ where m refers to the amount of melanin present and the proportion returned from the dermis as $R_d(c,h,\lambda)$, where c relates to the amount of collagen present and h haemoglobin: $I_r(\lambda)$, that proportion of light remitted from the skin can be described as $I_r(\lambda) = I_0(\lambda)A(m,\lambda)^2 R_d(c,h,\lambda)$. The $A(m,\lambda)^2$ term is due to light traversing the epidermis twice. The absorption of light by melanin $A(m,\lambda)$ can be shown to be an exponential term of the from $e^{m\alpha(\lambda)}$ where α is the absorption coefficient of melanin therefore resulting in:

$$I_r(\lambda) = I_0(\lambda)e^{2m\alpha(\lambda)}R_d(c,h,\lambda).$$

And

$$R_t(\lambda) = \frac{I_r(\lambda)}{I_0(\lambda)} = e^{2m\alpha(\lambda)}R_d(c,h,\lambda)$$ the ratio of light returned from the tissue

If $R_t(\lambda)$ is computed at different wavelengths and their natural logarithms then divided by one another $G(\lambda_1,\lambda_2)$ can be found where

$$G(\lambda_1,\lambda_2) = \frac{\ln(e^{2m\alpha(\lambda_1)}R_d(c,h,\lambda_1))}{\ln(e^{2m\alpha(\lambda_2)}R_d(c,h,\lambda_2))}$$

$\alpha(\lambda_1)$ and $\alpha(\lambda_2)$ are constants if λ_1 and λ_2 are fixed, so there exist a series of constants j and k where $2j\alpha(\lambda_1) = 2k\alpha(\lambda_2) = 1$ therefore there exists G' where

$$G'(\lambda_1,\lambda_2) = \frac{\ln(e^{2mjk\alpha(\lambda_2)}R_d(c,h,\lambda_1))}{\ln(e^{2mjk\alpha(\lambda_2)}R_d(c,h,\lambda_2))} = \frac{\ln\left(e^{m}R_d(c,h,\lambda_1)\right)}{\ln\left(e^{m}R_d(c,h,\lambda_2)\right)} = \ln(R_d(c,h,\lambda_1) - R_d(c,h,\lambda_2))$$

and therefore

$$Z = e^{G'} = R_d(c,h,\lambda_1) - R_d(c,h,\lambda_2)$$

f and k can easily be calculated by considering the absorption properties of melanin against wavelength or by experiment. The resulting term G' is independent of the melanin term being constructed solely from differences in the dermal component R_d. If wavelengths are then chosen where the haemoglobin term, h, is very small Z then becomes purely dependent on non-haemoglobin changes to the dermal component such as collagen and the presence of any other interesting material. Such wavelengths are easily accessible by silicon based sensors above approximately 600nm. It should therefore be possible construct images showing the variation of Z which may carry information pertinent to the structure of a skin lesion and in particular a BCC or SCC.

To test this hypothesis images of BCC's were acquired from 10 lesions including 5 superficial and 5 infiltrating/invasive . The wavelengths used included 700nm and 940nm at which the absorption of haemoglobin is negligible. Z was then computed across each lesion.

Two examples are shown in the accompanying figures, in which :-

Figure 1 shows a histologically confirmed superficial BCC with the Z image to the right. The Z image shows little difference between the surrounding tissue and the BCC.;

In contrast figure 2 shows an invasive BCC with its Z image indicating a marked difference from the surrounding tissue; and,

Figure 3 below shows an example computed at these shorter wavelengths showing the extent of collagen disruption

This pattern replicated itself through out all ten lesions with the invasive and infiltrating BCC's showing deviations on the Z image compared with the surrounding tissue whilst the superficial BCC's showed no such deviation.

The Z image construction and analysis produced information able to separate superficial from infiltrating and invasive BCC's. This information is important in the management of the most common form of cancer in human's allowing a clinician to treat superficial BCC's quickly and simply without surgery whilst ensuring that those that require surgery undergo a procedure with minimum delay. Another important consideration is that the technology required to implement this technique is readily available in the form of CCD and CMOS digital cameras although controlled illumination at specific wavelengths is required. This study only examined BCC's but it is a reasonable, although untested, hypothesis that a similar approach may yield information in the case of SCC's.

The analysis in this document specifically utilized near infrared wavelengths where the absorption of haemoglobin is low. This however limits the resolution of information relating to the disruption of collagen due to the cancer, if a lower frequency is used - for instance blue and green light - the spatial resolution of the collagen increases although there is artefact due to cross over with haemoglobin. This increase in resolution however appears to allow good discrimination of the edge of the cancer, something which is important in planning surgery, particularly Mohs surgery.

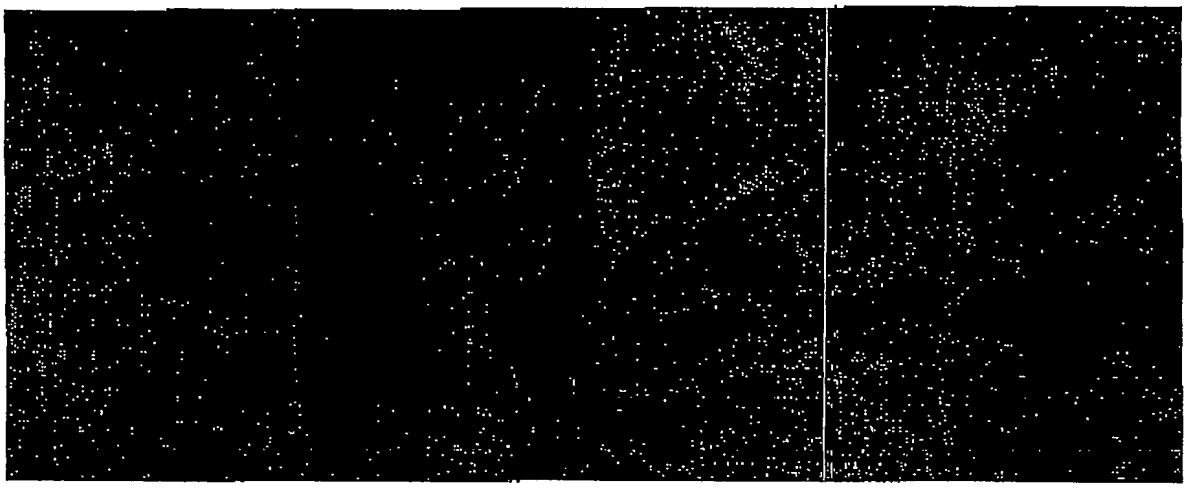

Figure 1 Superficial BCC with the Z image on the right showing no dermal involvement

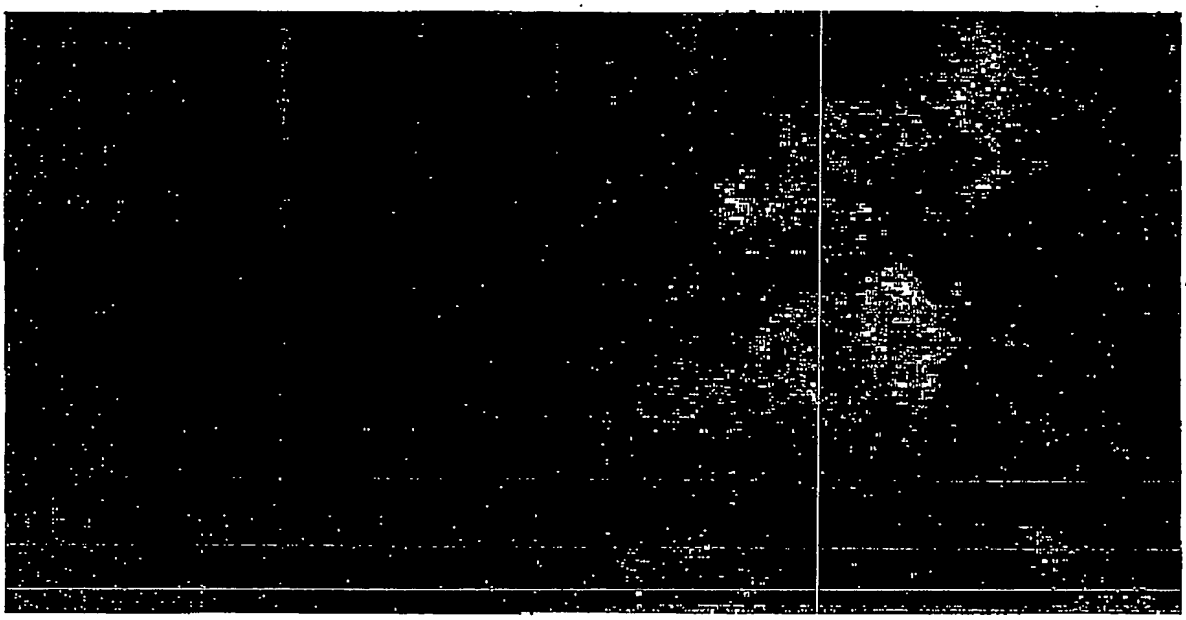

Figure 2 Invasive BCC with the Z image on the right showing marked dermal involvement

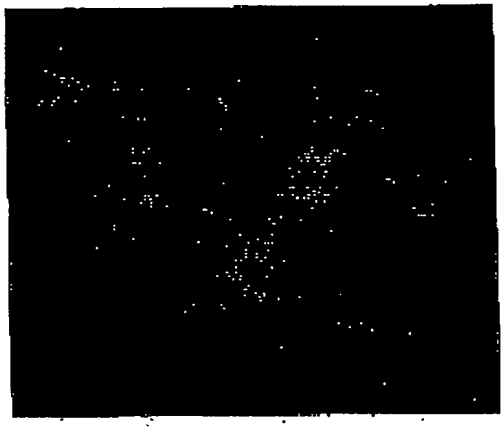

Figure 3 Collagen disruption showing in the Z value computed at shorter wavelengths

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☑ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☑ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.